Two 21bp dsDNA molecules are shown below. Which molecule will have the higher Tm if they were each placed in separate solutions with the same salt concentration. *Briefly explain your answer*. (10 points)

DNA molecule A: 5' ATAGCGTAGCTGTCGTATCGC 3'
3' TATCGCATCGACAGCATAGCG 5'

DNA molecule B: 5' GCGTAGGGCCGCTGCCTATAC 3'
3' CGCATCCCGGCGACGGATATG 5'

- Considering the DNA sequences only as they are written above, what could you do to make Molecule A's Tm equal to Molecule B's Tm

- In general terms, what two chemical interactions contribute to the stability of the DNA helical structure?

Use your knowledge about basic molecular biology techniques to determine the major products produced when the DNA substrate shown below is subjected to the following treatments. <u>After each treatment, write out the products produced</u>: (10 points)

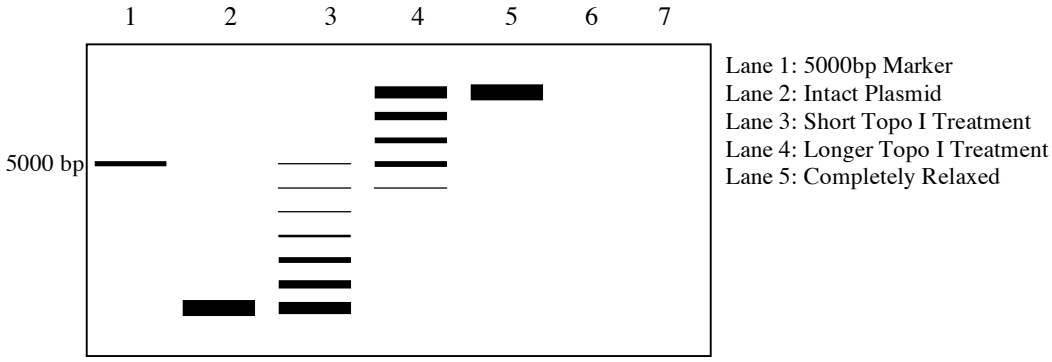
<u>DNA Substrate:</u> 5' AAAAACTG 3'
3' TTTTTGACGTATAGCG 5'

<u>Treatments are done in the following order:</u>
Step 1: DNA polymerase with all the dNTPS. The dCTP is <u>alpha</u> ^{32}P labeled deoxyCTP. This means that the alpha phosphate is the ^{32}P isotope

Step 2: Heat denaturation of the DNA products from step 1:

- ***Circle the products you have drawn in step 2 above that would contain the ^{32}P radiolabel***

You have a plasmid that is 5000bp in length. You carefully isolate this plasmid from *E.Coli* and find that it runs significantly faster (lane 2) than a linear 5000bp piece of marker DNA (lane 1).



You carefully mix your plasmid with *E. coli* topoisomerase I for varying amounts of time and run the results in lanes 3 and 4. The completely relaxed plasmid is shown in lane 5.

a. What is Lk° for this plasmid?

b. Explain why the intact plasmid runs faster than the linear 5000bp piece of DNA?

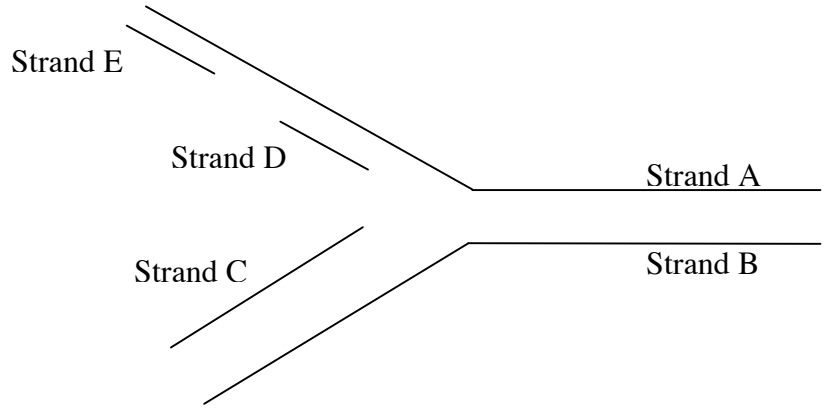
c. Using the information found in lanes 3-5 on the gel determine:

- Is the plasmid negatively or positively supercoiled?
- How many supercoils were present in the original intact plasmid you isolated?
- What was Lk for the original intact plasmid that you isolated?

d. You treat the intact plasmid you originally isolated with *E. coli* gyrase and ATP. Show in lane 6 where the plasmid would migrate on the gel.

e. You treat the intact plasmid you originally isolated with *E. coli* gyrase and no ATP. Show in lane 7 where the plasmid would migrate on the gel.

The diagram below shows one replication fork. Label the following on the diagram:



A. Label the 5' ends of all the DNA strands pictured

B. Which strand serves as the template for leading strand synthesis?

C. Which was synthesized first, strand D or E?

D. How is the gap between strands D and E closed? Be specific about the steps.

E. If a cell is treated with a chemical that inhibits the synthesis of UTP, why does that inhibit DNA replication if there is no U present in the DNA double helix?